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Award Number: W81XWH-08-2-0021

TITLE: Molecular Mechanisms Underlying Individual Differences in Response to Stress in a Previously Validated Animal Model of PTSD

PRINCIPAL INVESTIGATOR: Rachel Yehuda, PhD

CONTRACTING ORGANIZATION: Bronx Veterans Medical Research Foundation

Bronx, NY 10468-3904

REPORT DATE: September 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-09-2010	Annual	1 SEP 2009 - 31 AUG 2010

4. TITLE AND SUBTITLE

Molecular Mechanisms Underlying Individual Differences in Response to Stress in a Previously Validated Animal Model of PTSD

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-2-0021

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Rachel Yehuda, PhD
Joseph Buxbaum, PhD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Bronx Veterans Medical Research Foundation
Bronx, NY 10468-3904

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research And Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

It is unclear why some individuals develop PTSD while others do not. Pre-existing biological differences among individuals may predispose some and not others to develop PTSD. We are seeking to identify biological differences that may predispose to PTSD using an existing rat model for PTSD where exposure to a predator stress scent (PSS) results in extreme long-term behavioral responses for 25% of animals (EBR) thought to be analogous to "PTSD," while 25% of the animals show minimal behavioral responses (MBR). Our objectives for Experiment 1 are to identify genes showing altered expression in blood and brain areas implicated in PTSD that relate to individual differences in fear reactivity. Our goal in Experiment 2 is to examine the prophylactic effects of cortisol on the behavioral and gene expression effects of stress exposure. We have completed the behavioral work for Experiment 1 and successfully produced rats with the behavioral phenotypes (EBR and MBR) required for our gene expression aims. Brain and blood samples have been collected and the gene expression profiling has been completed on all brain samples and on a portion of the blood. Once gene expression analysis on the blood samples is complete, we will be able to conduct analyses examining the relationship between the changes in gene expression in the brain and blood in EBR, MBR and control rats. We will then repeat the study using a cortisol pretreatment condition.

15. SUBJECT TERMS

Posttraumatic stress disorder, animal models, gene expression

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	19	**19b. TELEPHONE NUMBER** *(include area code)*

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Annual Report for Army Award W81XWH-08-2-0021, entitled "Molecular Mechanisms Underlying Individual Differences in Response to Stress in a Previously Validated Animal Model of PTSD"

INTRODUCTION:

More than 1.5 million troops have been deployed to Iraq, and approximately 30% of returning Warfighters have post-traumatic stress disorder (PTSD). PTSD is a disorder that results from exposure to stressful events and results in significant and often long-term disability. It is at present unclear why some individuals exposed to stress develop PTSD while others exposed to similar challenges do not. One possibility is that pre-existing biological differences among individuals may predispose some and not others to develop PTSD. The goal of the present study is to identify potential biological differences that may predispose to PTSD using a validated PTSD rodent model that focuses on individual differences in response to stress. In this model, exposure to the scent of a predator (cat urine) results in long-term extreme behavioral responses for 25% of the animals thought to be analogous to PTSD and 25% of the animals show stress resistance (Cohen et al., 2004). Our overall objective is to identify genes showing altered expression in brain areas implicated in PTSD (anterior cortex, amygdala, and hippocampus) that relate specifically to individual differences in fear reactivity (Shin, Rauch & Pitman, 2006). We are also attempting to relate these brain changes in gene expression to those present in simultaneously obtained blood samples. Our final aim (Experiment 2) is to determine whether identified target genes are affected by a treatment that reduces stress reactivity - cortisol administration at the time of stress exposure (Moriceau et al., 2004; Schelling et al., 2006). We hypothesize that there will be different RNA expression profiles in the brains of rats showing extreme vs. minimal long-term behavioral responses to stress. We also expect that some genes differentially expressed in the brain will also differ in blood samples, but brain and blood may express unique markers associated with the behavioral phenotypes. We further hypothesize that some differences will no longer be present following cortisol pretreatment. These studies offer the opportunity to improve veteran's health by increasing our understanding of the biological basis of PTSD and may also suggest improved methods of treatment or prevention.

BODY:

During the previous year, extensive behavioral phenotyping was conducted as outlined in Appendix A. During the current year, brain and blood gene expression profiles in the animal groups were analyzed. Blood samples and brain tissue were processed for RNA isolation and gene expression profiling. Data analyses are now being conducted to identify differentially expressed genes across the animal groups.

Behavioral Methods and Findings for Experiment 1:

A PTSD rat model was generated using three behavioral classification groups that had been developed earlier the previous year: Extreme Behavioral Response (EBR), Partial Behavioral Response (PBR), and Minimal Behavioral Response (MBR), as well as non-

intervention controls (CON). The EBR rats were those who exhibited the most PTSD-like symptoms following exposure to predator stress scent (PSS), the PBR rats showed a partial response to PSS exposure, and the MBR rats were found to be well-adapted animals whose response to PSS exposure was minimal. Response were measured using the acoustic startle response paradigm and the elevated plus maze paradigm. The methodology was identical to that used with the first squad of rats earlier in the study. This second squad yielded an EBR group consisting of 12 female, and 13 male rats, a MBR group consisting of 12 female and 10 male rats, a PBR group consisting of 11 females and 13 males, and a control group consisting of 10 female and 10 male rats, forming a total of 91 animals.

Biological Specimens from Experiment 1:

Following completion of the behavioral work described above, animals were sacrificed and brain tissues were collected from five different brain areas: 1) Anterior Cortex, 2) Posterior Cortex, 3) amygdala, 4) hippocampus and 5) PAG (Periaqueductal gray). Peripheral whole blood was also collected from each animal.

- **Brain tissue and blood sample collections and RNA isolation**

Brain tissues from three different regions were collected, immediately kept in RNAlater solution (Ambion) following the manufacturer's manual to stabilize RNA, and then stored at − 80 °C. RNeasy Mini Kits (Qiagen) was used to isolate total RNA. On-column DNase digestion was used to remove DNA contamination. The purity and quality of the extracted total RNA were evaluated using the RNA 6000 LabChip kit and Agilent 2100 Bioanalyzer (Agilent Technologies). RNA concentrations were determined using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies). High quality RNA with RNA integrity numbers (RINs) greater than 8.0 was used for the microarray experiments.

At the time of the sacrifice, 2.5 ml whole peripheral blood from each subject was collected in PAXgene tube. Blood samples were incubated at room temperature for 4 hours for RNA stabilization and then stored at − 80 °C. RNA was extracted from whole blood using the PAXgene Blood RNA System Kit following the manufacturer's guidelines. RNA purification procedures followed the manufacturer's manual with the addition of on-column DNase digestion. Since there is a predominant presence of globin transcript in whole blood samples that constitute approximately 70% of mRNA, the globin depletion method was added to remove globin interference in the blood total RNA: the GLOBINclear™ Mouse/Rat kit (Ambion) was used to deplete globin mRNA from the blood total RNA samples

In brief, 2 µg of total RNA from whole blood was mixed with a biotinylated Capture Oligo Mix in hybridization buffer. The mixture was incubated for 15 minutes to allow the biotinylated oligonucleotides to hybridize with the globin mRNA species. Streptavidin magnetic Beads were then added to capture the globin mRNA. The magnetic beads were then pulled to the side of the tube with a magnet and the RNA, depleted of the globin mRNA, was transferred to a fresh tube. The RNA was further purified using a rapid magnetic bead-based purification method as suggested by the manufacturer. The RNA concentration and purity were determined. Only high quality RNA with RNA integrity numbers (RINs) greater than 8.0 was used for the microarray experiments.

- **Microarray Gene Expression**

Gene expression profiling was performed using the Illumina Rat Ref-12 Expression BeadChip Kit (Illumina) for genome-wide expression analysis. The BeadChip contains 21,910 probes selected primarily from the NCBI RefSeq database (Release 16). Illumina TotalPrep RNA Amplification Kit (Ambion) was used to do reverse transcription and in vitro transcription to synthesize cRNA from 200 ng RNA per sample. Briefly, double-stranded cDNA was synthesized using T7-oligo (dT) primers, followed by an in vitro transcription reaction to amplify cRNA while biotin was incorporated into the synthesized cRNA. After purification, the biotinylated cRNA was quantified using a NanoDrop spectrophotometer and the size distributions of cRNA assessed using Bioanalyzer. Then, 1.5µg biotinylated cRNA was hybridized to Rat Ref-12 Arrays (Illumina). The hybridization, washing, and scanning were performed according to the manufacturer's instructions. Microarray images were extracted automatically during the scanning. BeadStudio (Illumina) was used to normalize raw microarray intensity data.

- **Data analysis**

Data analysis is ongoing, using an in-house software suite derived from LIMMA (Linear Models for Microarray Data) and from open source bioinformatics software, Bioconductor. These methods are being used for clustering analysis and for detection of differentially expressed genes across the animal groups.

KEY RESEARCH ACCOMPLISHMENTS:

- Rat genome-wide expression on 90 brain samples using five subjects from each group: EBR, MBR, and CON, for both genders were completed.
- Genome-wide expression on 48 blood samples using eight subjects from the same three groups, for both genders, was completed.
- Preliminary data analysis shows differentially expressed genes between tissues and genders.
- No shared differentially expressed genes between males and females were observed in the brain samples
- We will conduct pathway analysis.
- We will also conduct TaqMan gene expression assays for further data validation.

REPORTABLE OUTCOMES:

There are no reportable outcomes to date.

CONCLUSION:

Gene expression analysis on the blood and brain samples from this squad is complete. While preliminary data analysis has shown the differential expression of genes between tissues and genders, we have not observed any shared differentially expressed genes between the two genders. This supports the presence of very distinct pathways for PTSD-like symptoms and behaviors between males and females. We intend to conduct further data analysis, namely pathway analysis using Ingenuity Pathway Analysis software to interpret the many changes. We also intend to perform TaqMan gene expression assays for further data validation. This will allow us to achieve our aim for Experiment 1 of identifying genes that are differentially

expressed in the blood and three brain regions associated with fear responses in rats that develop PTSD-like symptoms, and those that are resistant to developing these symptoms. We will then repeat the study using a cortisol pretreatment condition. It is hoped that the results obtained from this work will increase our understanding to the biology of PTSD and potentially facilitate the detection of PTSD susceptibility and the treatment of the disorder.

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Appendix A

Year 1 procedures and progress:

During the first year, the behavioral work for our first proposed experiment examining brain and blood gene expression profiles in animals that display extreme long-term responses to a stressor or a minimal behavioral response was completed. Blood samples and brain tissue were also obtained, brain micro dissections were performed, and gene expression profiling was conducted on the brain tissue samples.

Behavioral Methods and Findings for Experiment 1:

Animals underwent behavioral testing in two squads. The methods and results for the male and females in the first squad of animals are presented in detail, and are representative of our overall behavioral findings. The results for the second squad are summarized below.

The behavioral results obtained from the first squad of animals tested for Experiment 1 confirmed that the stress paradigm reliably produces three distinct behavioral phenotypes in both male and female rats. The behavioral responses of 28 adult male and 28 adult female Sprague-Dawley rats (an out-bread strain) were examined.

Male Rats

Male rats were randomly assigned to two groups: Naive control rats (n-5) were exposed to fresh, unused cat litter for 10 min while rats in the stress exposed condition (n=23) were exposed to a predator scent (cat urine) for 10 min. Behavioral responses were assessed 7 days after predator stress scent (PSS) exposure. The behavioral paradigms used were the acoustic startle response (ASR) paradigm and the elevated plus maze (EPM). In the latter paradigm rats are placed on an elevated maze containing both covered and uncovered arms. Anxiety/fear is quantified by assessing the relative time rats spend in the covered and uncovered arms, with an increased proportion of time spent in covered arms indicating increased fear/anxiety. The key variables of interest in the acoustic startle paradigm are startle amplitude following exposure to a 110 dB sound burst, and the degree to which the startle response declines over multiple trials (startle habituation). Both the EPM and ASR have been shown to be a valid measure of stress-response (Adamec, Shallow & Budgell, 1997; Garrick et al, 1997), and behavior in both paradigms is easily quantified.

The assessment of behavioral phenotypes was performed in two steps. We first performed a preliminary data assessment to verify that PSS had a significant behavioral effect on the exposed rats as a group, and elicited a range of individual responses. Cutoff behavioral criteria (CBC) incorporating fearful behavior on the EPM and non-habituated/exaggerated ASR were then used to divide the PSS-exposed rats into three behavioral phenotypes: extreme behavioral responders (EBR), partial behavioral responders (PBR) and minimal behavioral responders (MBR). To maximize resolution and minimize false positives extreme responses on both the EPM and ASR were required for inclusion in the EBR group (anxious, fearful and hyper-vigilant, i.e. PTSD-like symptoms), whereas a negligible degree of response to both was required for inclusion in the MBR group. The validity of the criteria have been previously verified by ascertaining that the vast majority (>90%) of unexposed control animals conform to the criteria for MBR (unaffected by test procedures) and less than 3%, meet the criteria for EBR (Cohen et al., 2006). The cut-off criteria were as follows:

EBR:

1. Five minutes (entire session) spent in closed arms and no entries into the open-arms on the EPM.
2. Mean startle amplitude > 700 units and no habituation over time.

MBR:

1. One minute or less time spent in closed arms and eight or more open-arm entries on the EPM.
2. Mean startle amplitude < 700 units and habituation of the response over trials.

PBR:

1. Behavioral responses intermediate to those above.

In keeping with the work of Blanchard and Blanchard (Blanchard et al., 1990; Blanchard et al., 1998; Blanchard et al., 1993), Adamec (Adamec, 1997; Ademec et al., 1998; Adamec, Blundell & Burton 2003; Adamec et al, 1999; Adamec & Shallow, 1993) and Cohen (Cohen et al., 1996; Cohen, Kaplan & Kotler, 1999; Cohen & Zohar, 2004; Cohen, Zohar and Matar, 2003; Cohen et al., 2005; Cohen et al. 2004), the proportion of individuals displaying behaviors fulfilling criteria for EBR at 7 days post PSS exposure were considered to demonstrate long-term and persistent behavioral change. Beyond this time frame significant changes in the prevalence of EBR are not observed.

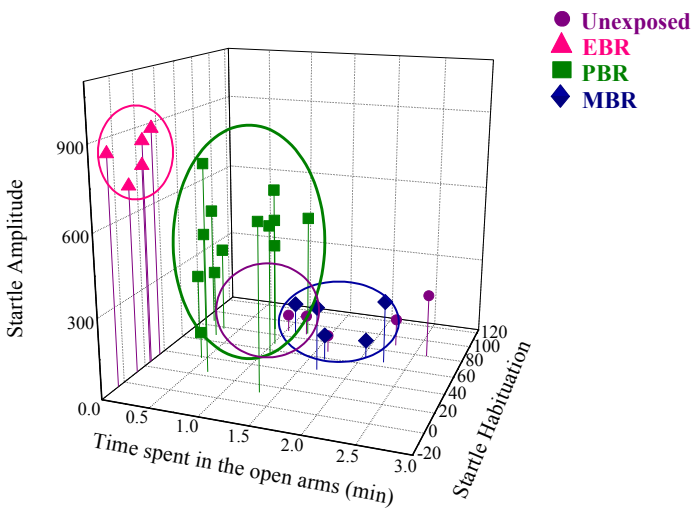

Figure 1. *The elevated plus maze (EPM) behavior and the acoustic startle responses of male rats classified as extreme behavioral responders (EBR), partial behavioral responders (PBR)minimal behavioral responders (MBR) or unexposed controls one week after a 10 min exposure to predator stress scent (cat urine). The X-axis represents time spent in the open arms of the EPM, the Y-axis represents acoustic startle amplitude, and the Z axis represents startle habituation.*

PSS-exposed male rats exhibited highly variable behavioral responses one week following PSS exposure and EBR, PBR and MBR subgroups were identifiable (see Fig. 1). The graphic representation of the data from both paradigms (EPM and ASR) reveals two rather distinct features. First, it is clear that PSS exposure alters the response of the majority of individuals to at least some degree. A single ten-minute exposure to PSS

significantly increased anxiety-like avoidance of open spaces as compared to unexposed controls. Values for time spent in the open arms ($F_{(1,26)} = 8.4$, $p < .0075$) were significantly decreased after stress exposure, as compared to control conditions. It is unlikely that a more general impairment of locomotion/exploration underlies these results as there were no differences in total exploration of the maze between groups. PSS exposure also significantly increased mean startle amplitude and caused a significant deficit in ASR habituation in exposed rats as compared to controls ($F_{(1,26)} = 4.8$, $p < .004$ and $F_{(1,26)} = 8.2$, $p < .0085$ respectively).

When classified using the CBCs, five PSS-exposed rats (21.8%) fulfilled criteria for EBR, whereas 5 rats (21.8%) were characterized as MBR. All other rats fell between the CBC's for the extreme groups, and were defined as PBR. In the control group no rats (0%) fulfilled criteria for EBR.

Female Rats

Female rats were also randomly assigned to two groups: Naive control rats (n-5) were exposed to fresh, unused cat litter for 10 min while rats in the stress exposed condition (n=24) were exposed to the PSS for 10 min. Behavioral responses were assessed 7 days later and behavioral classification was performed using the methods described above.

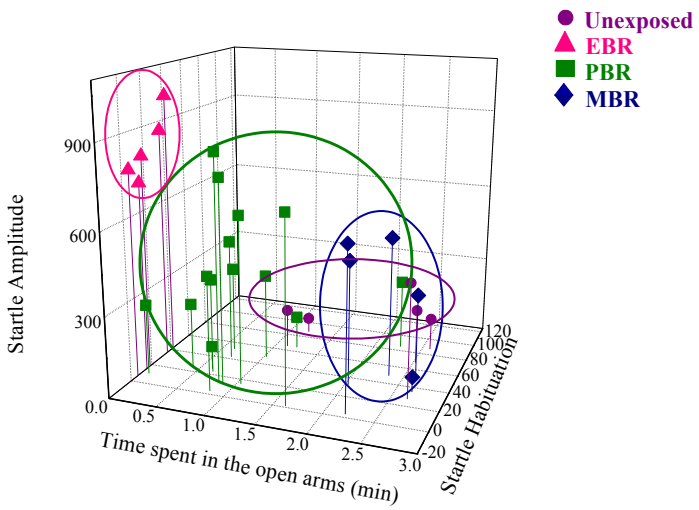

Figure 2. *The elevated plus maze (EPM) behavior and the acoustic startle responses of female rats classified as extreme behavioral responders (EBR), partial behavioral responders (PBR)minimal behavioral responders (MBR) or unexposed controls one week after a 10 min exposure to predator stress scent (cat urine). The X-axis represents time spent in the open arms of the EPM, the Y-axis represents acoustic*

Similar to males, PSS-exposed female rats exhibited highly variable behavioral responses and EBR, PBR and MBR subgroups were identifiable (see Fig. 1). The time spent by females in the open arms of the EPM was also significantly decreased one week after PSS exposure compared to controls ($F_{(1,27)} = 5.2$, $p < .035$), and there were no differences in total exploration of the maze between groups. As was the case in males, PSS exposure also significantly increased the mean startle amplitude of females and reduced habituation of the ASR. ($F_{(1,27)} = 12.1$, $p < .002$ and $F_{(1,27)} = 6.03$, $p < .025$ respectively).

Based on the CBCs,

five PSS-exposed females (20.8%) fulfilled criteria for EBR, whereas five (20.8%) were characterized as MBR. All other PSS-exposed females were PBR. No control females (0%) fulfilled criteria for EBR.

The second squad of animals underwent behavioral testing and were classified using the methods described above and yielded a second EBR group consisting of 12 female, and 13 male rats, a second MBR group consisting of 12 female and 10 male rats and a second control group consisting of 10 female and 10 male rats.

Biological Specimens from Experiment 1:

Following completion of the behavioral work described above, animals were sacrificed, trunk blood samples were collected, and brain micro-dissection was performed to isolate the anterior cortex (AC), amygdale (AM), and hippocampus (HI).

Three brain tissue samples (AC, AM and HI) from each rat classified as EBR, MBR or a control animal above have undergone genome-wide gene expression profiling using Illumina BeadArrays on the Sentrix Platform.

An example of the analyses performed on the data to date is presented below for the first squad of animals to undergo behavioral testing. For this squad of animals there were 5 female and 5 male EBR, 5 female and 5 male MBR and 5 female and 5 male control rats. The analysis was conducted using a 3 Tissues X 3 Behaviors X 2 Sexes block design with 5 different rats for each combination of Sex and Behavior as is presented schematically in Figure 3.

		RNA sample ID (gene expression)		
	Rat #	AC	AM	HI
female CON	F1	1	11	21
	F2	2	12	22
	F3	3	13	23
	F4	4	14	24
	F5	5	15	25
female MBR	F27	31	43	55
	F26	32	44	56
	F24	33	45	57
	F22	34	46	58
	F21	35	47	59
female EBR	45	67	79	91
	44	68	80	92
	F36	69	81	93
	F39	72	84	96
	F40	73	85	97
male MBR	43R	103	113	123
	32R	104	114	124
	30R	105	115	125
	20R	106	116	126
	11R	107	117	127
male EBR	2R	133	146	159
	8R	134	147	160
	9R	135	148	161
	10R	136	149	162
	12R	137	150	163
male CON	2R	172	182	192
	4R	173	183	193
	5R	174	184	194
	6R	175	185	195
	7R	176	186	196
repeated		1	11	21
repeated		172	182	192

CON: Control
MBR: Well-adapted animal
EBR: PTSD-like

AC: Anterior cortex
AM: Amygdala
HI: Hippocampus

Figure 3: *Schematic representation of study design.*

Quality Controls & Normalization:

From the raw data provided, we extracted the probe signal values and the probe detection p-values and consolidated each into a 22,523 X 96 matrix. We then log2 transformed the exponentially distributed data and saw that most of the data was in the lower (noise) end of the distribution (see Fig. 4).

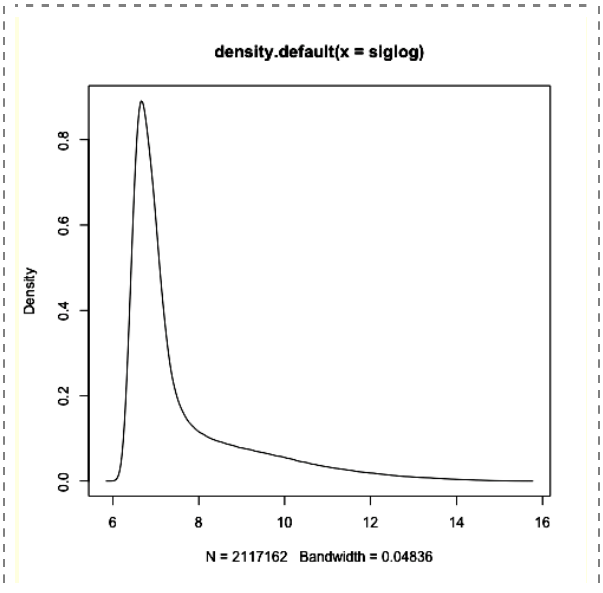

Figure 4: *Signal Distribution (Log2 Scale).*

We then examine the detection p-values. For each chip, we counted the number of probes that are detected at a significance level of $p < 0.05$ and then plotted the counts across chips. As is depicted in Figure 5, most of the chips are clustered around 10000 or so genes expressed. We also note two chips (samples 137 and 163) that show only 2000 or so genes expressed by this metric. We removed these chips from further analysis.

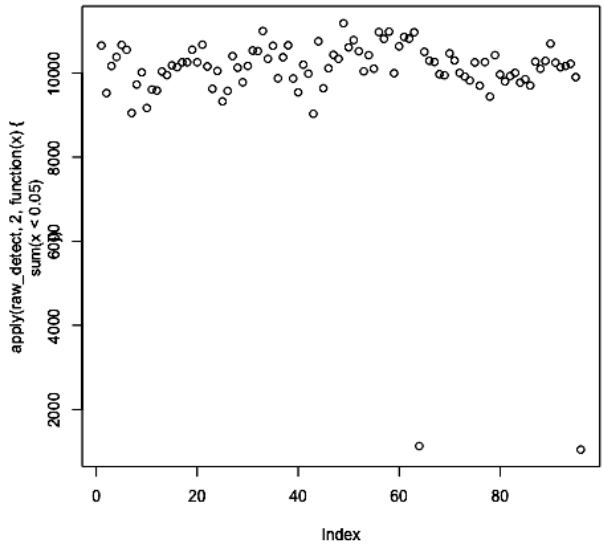

Figure 5*: Number of probes present per chip ($p < 0.05$)*

After we removed the outliers, we checked to see if normalization of the data set was warranted. We plotted the means of all the chips and observed that there was a non-trivially wide spread (2-4 fold difference). We therefore opted for quantile normalization and observed that chip-wise distributions were now identical (see Fig. 6).

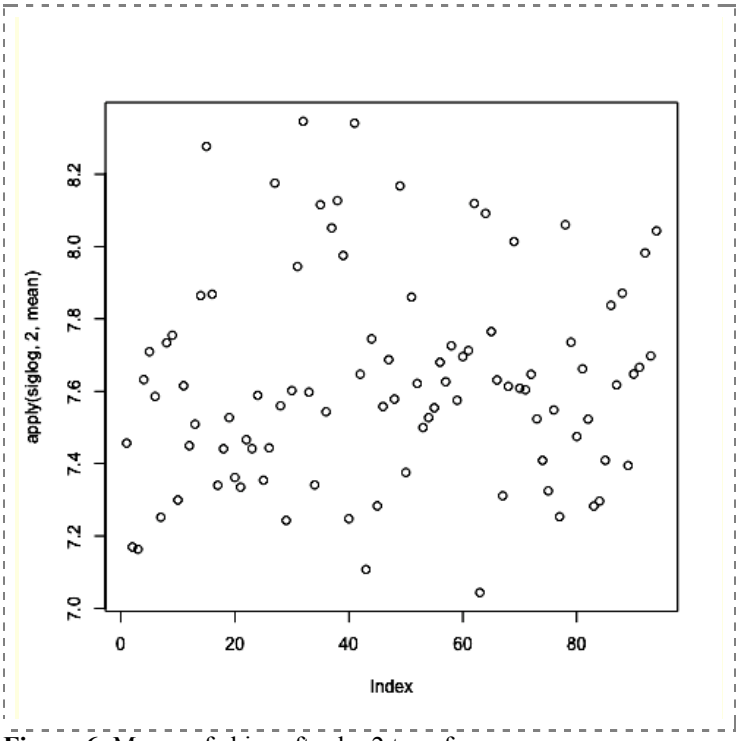

Figure 6: Means of chips after log2 transform

Since we are mainly interested in large scale expression changes, we then used the detection p-values to remove genes that were poorly expressed in the dataset. To do this we averaged the detection p-values across chips for each probe. We retained a probe if its average detection p-value was below 0.05. We were then left with 9092 genes that were well expressed in our data set.

Replicate Clustering:

After the preliminary data curation was completed, we looked at the general structure of the data. This helps answer questions such as:

- Do my replicates cluster together?
- Are the differences between tissues dominant or secondary?
- Are my control and experimental conditions easily separable?

For a first pass, we use multi-dimensional scaling to project the chip-wise correlation matrix into two dimensions (see Fig. 7). After a Sammon transform we achieved a reasonable projection (stress < 0.164) into a 2-D mds space. We see that some replicate sets are very tightly clustered while others are more variant. We also see large differences between males (right side, triangles) and females (left side, circles) that seem to dominate the differences between amygdala and anterior cortex.

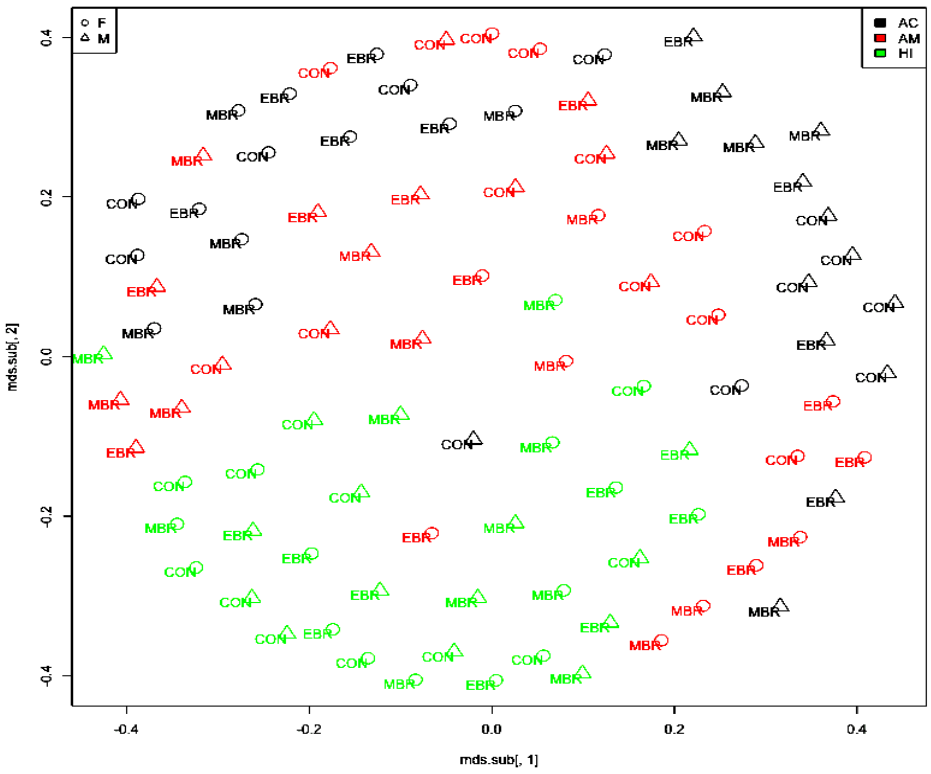

Figure 7: *MDS projection of chip-wise sp correls.*

Differential Expression Analysis:

As a first step in examining differential expression analysis, we sought to see if simple linear models could sufficiently explain the experimental variables. To do this more simply, we broke the experiment down by tissue type and for each set, we formulated a 2-factor linear model in R using the limma package. Specifically:

1. We parameterized the model according to Sex*Response.
2. We used the block design of the individual rats to estimate the duplicate correlation.
3. We fed the model, the blocks, and the correlations to limma to estimate coefficients.
4. We formulated contrasts to pull out EBR vs CON and MBR vs CON in each of the sexes
5. We fit the contrasts to the model and estimated significance for each fit.
6. We adjusted for multiple testing and were left with sets of differentially expressed genes

The limma contrasts just provide us with 4 differentials (F:EBRvCON, F:MBRvCON, M:EBRvCON, M:MBRvCON) -- the first 4 in the result matrix below. To quickly pick out all intersections and "outer" sections of the pairs of contrasts we added the following columns:

- FandM_EBR-CON: Differences from EBR-CON shared by the sexes.
- FandM_MBR-CON: Differences from MBR-CON shared by the sexes.
- F_EBR&MBR: Where EBR and MBR behave alike in females.
- M_EBR&MBR: Where EBR and MBR behave alike in males.
- F_EBRnotMBR: Changes exclusive to EBR in females.
- M_EBRnotMBR: Changes exclusive to EBR in males.
- F_MBRnotEBR: Changes exclusive to MBR in females.
- M_MBRnotEBR: Changes exclusive to MBR in males.

Differences within Tissues**:**

The numbers of genes found differentially expressed in each of these contrasts can be found in the table below which depicts the number of repressed (Rprsd) genes and induced (Indcd) genes in three different brain areas anterior cortex (AC), amygdale (AM), and hippocampus (HI). It is interesting to note that there are no shared differentially expressed genes between males and females in this set. There also seem to be tissue specific biases between the sexes such as no genes changing in M:EBR-CON in the AM set or the HI set.

AC	F_EBR-CON	F_MBR-CON	F_EBR-MBR	M_EBR-CON	M_MBR-CON	M_EBR-MBR	FandM_EBR-CON	FandM_MBR-CON	FandM_EBR-MBR	F_EBRandMBR	M_EBRandMBR	F_EBRnotMBR	M_EBRnotMBR	F_MBRnotEBR	M_MBRnotEBR
Rprsd -1	162	256	0	86	32	0	18	3	0	106	24	56	62	150	8
Unchd 0	8738	8588	9092	8917	9034	9092	9061	9078	9092	8885	9062	8945	8947	8795	9064
Indcd +1	192	248	0	89	26	0	13	11	0	101	6	91	83	147	20

AM	F_EBR-CON	F_MBR-CON	F_EBR-MBR	M_EBR-CON	M_MBR-CON	M_EBR-MBR	FandM_EBR-CON	FandM_MBR-CON	FandM_EBR-MBR	F_EBRandMBR	M_EBRandMBR	F_EBRnotMBR	M_EBRnotMBR	F_MBRnotEBR	M_MBRnotEBR

Rprsd -1	297	28	9	0	54	0	0	3	0	19	0	278	0	9	54
Unchd 0	8453	9028	9075	9092	8996	9092	9092	9089	9092	9045	9092	8500	9092	9075	8996
Indcd +1	342	36	8	0	42	0	0	0	0	28	0	314	0	8	42

HI	**F_EBR-CON**	**F_MBR-CON**	**F_EBR-MBR**	**M_EBR-CON**	**M_MBR-CON**	**M_EBR-MBR**	**FandM_EBR-CON**	**FandM_MBR-CON**	**FandM_EBR-MBR**	**F_EBRandMBR**	**M_EBRandMBR**	**F_EBRnotMBR**	**M_EBRnotMBR**	**F_MBRnotEBR**	**M_MBRnotEBR**
Rprsd -1	44	0	241	0	46	3	0	0	1	0	0	44	0	0	46
Unchd 0	8983	9092	8715	9092	9027	9084	9092	9092	9090	9092	9092	8983	9092	9092	9027
Indcd +1	65	0	136	0	19	5	0	0	1	0	0	65	0	0	19

Genome –wide gene expression analysis has now been conducted on the brain samples from both squads of rats that underwent behavioral testing, and the blood samples are currently being analyzed. Once the gene expression data have been obtained from the blood samples (which we view as the most potentially useful source for clinically relevant biomarkers), we will be able to conduct a thorough analyses examining the relationship between the changes in gene expression in the brain and blood in EBR, MBR and control rats thereby achieving our aims for Experiment 1.

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